that promote meiotic synthesis dependent strand annealing remain unknown in any organism (Figure 1).

Overall, the Mets and Meyer study reveals that crossover formation in *C. elegans* is controlled at two levels: at the level of meiotic DSB production by a new condensin complex, and at the level of the crossover/noncrossover decision, the control and execution of which remains to be defined.

REFERENCES

Bishop, D.K., and Zickler, D. (2004). Cell *117*, 9–15.

Blat, Y., and Kleckner, N. (1999). Cell *98*, 249–259.

Csankovszki, G., Collette, K., Spahl, K., Carey, J., Snyder, M., Petty, E., Patel, U., Tabuchi, T., Liu, H., McLeod, I., et al. (2009). Curr. Biol. *19*, 9–19.

Gerton, J.L., DeRisi, J., Shroff, R., Lichten, M., Brown, P.O., and Petes, T.D. (2000). Proc. Natl. Acad. Sci. USA *97*, 11383–11390.

Hillers, K.J., and Villeneuve, A.M. (2003). Curr. Biol. *13*, 1641–1647.

Kelly, K.O., Dernburg, A.F., Stanfield, G.M., and Villeneuve, A.M. (2000). Genetics *156*, 617–630.

Maloisel, L., and Rossignol, J.L. (1998). Genes Dev. *12*, 1381–1389.

Mets, D.G., and Meyer, B.J. (2009). Cell, this issue.

Tsai, C.J., Mets, D.G., Albrecht, M.R., Nix, P., Chan, A., and Meyer, B.J. (2008). Genes Dev. *22*, 194–211.

It's All about Timing

Gary T. Philips[1] and Thomas J. Carew[1,*]
[1]Department of Neurobiology and Behavior and the Center for the Neurobiology of Learning and Memory, University of California, Irvine, Irvine, CA 92607-4450, USA
*Correspondence: tcarew@uci.edu
DOI 10.1016/j.cell.2009.09.020

In the formation of long-term memories, a "spaced" distribution of study sessions is more beneficial than closely spaced "massed" study sessions. Pagani et al. (2009) examine the molecular basis of this spacing effect in *Drosophila* and find a role for the SHP2 homolog, corkscrew, an activator of Ras/MAPK signaling, in establishing optimal spacing intervals.

Increasing the amount of time spent studying improves memory retention, but the distribution of study sessions across time is equally critical for memory formation. The *spacing effect* refers to the benefit to enduring memory retention of a "spaced" distribution of study sessions compared to a continuous study session of the same total duration, or more closely spaced "massed" sessions. Although the benefits of this spacing effect in both humans and animal models have been known for over a century, the underlying molecular mechanisms are still poorly understood. From studies in a wide range of experimental systems, we now have an extensive list of candidate molecules and cellular correlates that can, at least in principle, contribute to this sensitivity to training patterns (Figure 1). Recent work has implicated the Ras/MAPK pathway in regulating the optimal spacing intervals for long-lasting memory formation (Ajay and Bhalla, 2004; Philips et al., 2007; Ye et al., 2008). In this issue of *Cell*, Pagani et al. (2009) characterize a role in long-lasting memory formation for a *Drosophila* tyrosine phosphatase called corkscrew (SHP2 in vertebrates), a potent activator of Ras/MAPK signaling. They show that corkscrew activity regulates the appropriate training intervals for the induction of long-term memory in flies.

Memory formation in *Drosophila* is sensitive to both the number and pattern of training sessions. In response to multiple spaced training sessions, two forms of enduring memory can be formed. One type of memory does not require protein synthesis and lasts about 4 days (also called anesthesia-resistant memory). A second type of memory, long-term memory, lasts at least 1 week and requires both protein synthesis and CREB-dependent gene transcription. The Ras/MAPK signaling pathway, which regulates many cellular processes, also plays a role in the formation of long-term memory, through its effects on both protein synthesis and CREB-dependent transcription.

The SHP2 tyrosine phosphatase is an activator of the Ras/MAPK pathway. In humans, dominant mutations in the gene encoding SHP2, *ptpn11*, are associated with the development of Noonan's and LEOPARD syndromes. These syndromes belong to a family of Ras/MAPK-related disorders associated with mental retardation. Most clinically relevant mutations in *ptpn11* are associated with prolonged SHP2 phosphatase activity that promotes the conversion of the MAPK activator Ras from its inactive state to its active state. Thus, gain-of-function SHP2 mutants lead to prolonged activation of the Ras/MAPK pathway.

In their new work, Pagani et al. (2009) examine the role of corkscrew, the fly homolog of SHP2, in the formation of long-term memory. The authors use a common aversive olfactory memory task, in which flies are first given an electric shock in the presence of a specific odor. Later, they demonstrate memory for that experience in a two-choice apparatus by avoiding a chamber containing the odor

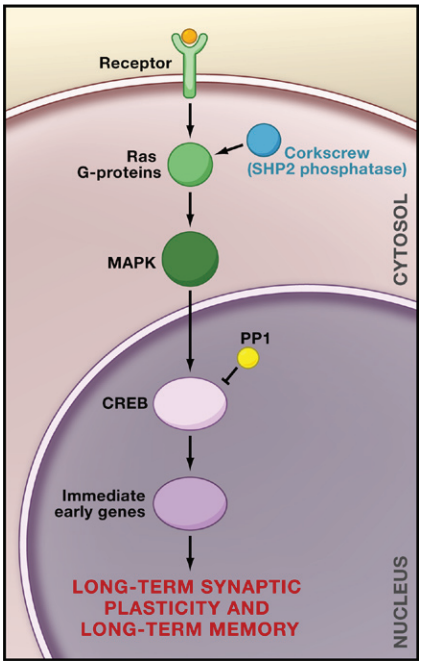

Figure 1. Signaling Elements Contributing to the Spacing Effect in Memory Formation
Illustrated are some of the candidate signaling elements implicated in sensitivity to training patterns during the induction of long-term synaptic plasticity and memory. Ras signaling (Ye et al., 2008) promotes MAPK activation (Ajay and Bhalla, 2004; Philips et al., 2007), whose activity can, in turn, support the activation of nuclear signaling components such as the CREB transcription factor (Yin et al., 1995; Kogan et al., 1997; Josselyn et al., 2001). CREB transcriptional activity leads to the expression of immediate early genes (Guzowski, 2002) and subsequent production of the proteins necessary to support long-term synaptic plasticity and long-term memory formation. Massed training sessions can recruit inhibitory phosphatases, including the CREB-inactivating protein phosphatase 1 (PP1), which serves as a "brake" on the formation of long-term synaptic plasticity and memory (Muzzio et al., 1999; Genoux et al., 2002). Pagani et al. (2009) now show that corkscrew (blue), the *Drosophila* homolog of the Ras/MAPK activating protein SHP2, can modulate the optimal spacing intervals for long-term memory formation in flies.

paired with a shock in favor of a chamber containing an explicitly unpaired "safe" odor. In this task, long-term memory typically develops after ten spaced training sessions. Corkscrew is constitutively expressed in wild-type flies. When the authors overexpressed gain-of-function mutant forms of the *corkscrew* gene in the fly central nervous system, which result in prolonged corkscrew phosphatase activity, they discovered that long-term memory formation was disrupted. In contrast, overexpression of corkscrew mutants did not affect anesthesia-resistant memory in these flies. The investigators observed long-term memory disruption both in transgenic flies overexpressing mutant corkscrew from birth and in flies overexpressing mutant corkscrew just 1 hr prior to training. Thus, impaired long-term memory formation in this fly model of Noonan's syndrome suggests a direct role for corkscrew in memory formation, rather than a developmental role with indirect effects on memory. Importantly, the authors also demonstrated, using both RNA interference against corkscrew and a pharmacological inhibitor of corkscrew phosphatase activity, that endogenous corkscrew activity is normally required for long-term memory induction.

In the course of their study, the authors made an intriguing observation. Flies that overexpressed wild-type corkscrew (in which long-term memory formation was normal following spaced training) now also developed a protein synthesis-dependent 24 hr long-term memory when given a massed training protocol. Massed training typically only induces anesthesia-resistant memory. Therefore, overexpression of wild-type corkscrew enabled training with significantly shorter rest intervals between trials (2.5 versus 15 min) to induce a 24 hr long-term memory. The induction of long-term memory required corkscrew's phosphatase activity, as overexpression of a phosphatase-defective mutant did not yield the same results. Thus, although overexpression of gain-of-function corkscrew impaired long-term memory formation, overexpression of wild-type corkscrew actually promoted long-term memory formation during massed training sessions. These data strongly suggest that the memory deficit observed in flies overexpressing the gain-of-function corkscrew mutations may be due to the decreased ability to inactivate corkscrew phosphatase activity.

As corkscrew is a potent activator of Ras/MAPK signaling, which is required for long-term memory induction in both vertebrate and invertebrate systems, the authors measured MAPK activation in flies undergoing ten spaced or massed training sessions. MAPK signaling was briefly activated in control flies following both spaced and massed training protocols. However, although the authors observed significant MAPK activation between each of the ten spaced training trials, they did not observe any MAPK activation between massed training sessions. In contrast, the authors did observe MAPK activation in flies undergoing massed training if the flies overexpressed wild-type corkscrew (recall that massed training induces long-term memory in these flies). Thus, successful induction of long-term memory appears to be correlated with MAPK activity during the intervals between training trials.

The authors' most striking observation, however, was that during successful long-term memory induction protocols, administration of the second training trial resulted in significant inactivation of the MAPK that had been activated by the first trial. In contrast, a second trial in flies overexpressing gain-of-function corkscrew mutant protein (which impairs long-term memory formation) did not inactivate MAPK. The authors therefore suggest that it is the ability of a subsequent training trial to turn off (and then back on) MAPK activation—that is, the generation of discrete waves of MAPK activation—that plays an important role in long-term memory induction. This is a new idea as previous research had correlated the optimal training intervals for induction of long-term synaptic plasticity and memory with the timing of peak MAPK activity between trials (Ajay and Bhalla, 2004; Philips et al., 2007) but had not considered the trial-to-trial dynamic regulation of MAPK activity. Finally, the authors rescued the long-term memory deficits of gain-of-function *corkscrew* mutants both pharmacologically, by reducing corkscrew phosphatase activity prior to spaced training, and also without drug intervention, by extending the interval between training sessions from 15 to 40 min. As control flies also learned normally on the 40 min interval protocol, these results collectively suggest that the phosphatase activity of corkscrew regulates the minimum inter-trial interval required for successful long-term memory formation. The authors propose that expanding the intervals between training sessions to 40 min in flies overexpressing gain-of-function corkscrew mutants rescues long-term memory formation by permitting MAPK inactivation with sub-

sequent training (which cannot occur at the shorter spacing intervals). This proposal underscores the importance of the generation of discrete waves of MAPK activity with each trial.

In summary, the authors have demonstrated the importance of activation and inactivation of MAPK in the first 2 (out of 10) training trials leading to long-term memory formation in flies. To explore this model further, it will now be important to test whether a similar on-off switch for MAPK activity occurs across trials 3–10 in normal flies, and in gain-of-function *corkscrew* mutant flies trained using the 40 min interval protocol. If these predictions are confirmed, this significantly advances our understanding of the spacing effect, as it indicates that it is not only the activation kinetics of MAPK signaling that determine the optimal spacing of training sessions, but also the generation of discrete waves of MAPK that is critical. Ultimately, an understanding of such activation profiles in patients suffering from disorders of the Ras/MAPK signaling pathway such as Noonan's syndrome could, in principle, lead to the development of optimal learning strategies that would allow the encoding of lasting memories.

REFERENCES

Ajay, S.M., and Bhalla, U.S. (2004). Eur. J. Neurosci. *20*, 2671–2680.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D., and Mansuy, I.M. (2002). Nature *418*, 970–975.

Guzowski, J.F. (2002). Hippocampus *12*, 86–104.

Josselyn, S.A., Shi, C., Carlezon, W.A., Jr., Neve, R.L., Nestler, E.J., and Davis, M. (2001). J. Neurosci. *21*, 2404–2412.

Kogan, J.H., Frankland, P.W., Blendy, J.A., Coblentz, J., Marowitz, Z., Schutz, G., and Silva, A.J. (1997). Curr. Biol. *7*, 1–11.

Muzzio, I.A., Ramirez, R.R., Talk, A.C., and Matzel, L.D. (1999). Behav. Neurosci. *113*, 103–117.

Pagani, M.H., Oishi, K., Gelb, B.D., and Zhong, Y. (2009). Cell, this issue.

Philips, G.T., Tzvetkova, E.I., and Carew, T.J. (2007). J. Neurosci. *27*, 13701–13705.

Ye, X., Shobe, J.L., Sharma, S.K., Marina, A., and Carew, T.J. (2008). Proc. Natl. Acad. Sci. USA *105*, 20511–20516.

Yin, J.C., Del Vecchio, M., Zhou, H., and Tully, T. (1995). Cell *81*, 107–115.

DNA Double-Strand Breaks Come into Focus

Karl-Peter Hopfner[1,*]

[1]Center for Integrated Protein Science Munich and Munich Center for Advanced Photonics at the Gene Center, Department of Chemistry and Biochemistry, Ludwig-Maximilians-University Munich, 81377 Munich, Germany

*Correspondence: hopfner@lmb.uni-muenchen.de

DOI 10.1016/j.cell.2009.09.017

The Mre11-Rad50-Nbs1 (MRN) complex senses DNA double-strand breaks and recruits different repair pathway and checkpoint proteins to break foci. Two new studies (Williams et al., 2009; Lloyd et al., 2009) identify Nbs1 as a key factor in this process and reveal how an N-terminal protein recruitment module in Nbs1 binds to different response factors through shared phosphopeptide motifs.

Of the various types of DNA damage, double-strand breaks (DSBs) may be the most cytotoxic because of their potential to cause gross chromosomal aberrations, often linked to cell death or cancer. Cells therefore go to great lengths to repair DSBs, mounting a highly complex multistep response that includes modifications to large chromatin domains ("repair foci") through, e.g., ubiquitination, phosphorylation, and binding of numerous repair factors, scaffolding mediators, and posttranslational modifiers (Harper and Elledge, 2007). A keystone in the response to DSBs in eukaryotic cells is the Nbs1 protein, one of the earliest repair factors to bind to DSBs. However, Nbs1 also acts later in the repair process to regulate the DNA damage checkpoint and to recruit other repair factors to DSBs. Two papers in this issue of *Cell* (Williams et al., 2009; Lloyd et al., 2009) now provide structural and molecular insight into the mechanism by which Nbs1 performs these later repair functions.

DSBs are repaired by two major pathways, homologous recombination (HR) and nonhomologous end joining (NHEJ). Homologous recombination uses the sister chromatid as a template for new DNA synthesis and is highly accurate but limited to the S and G2 phases of the cell cycle. In NHEJ, in contrast, DNA ends are directly ligated without the need for sister chromatids, but this repair pathway is potentially mutagenic and can lead to chromosome aberrations. Repair pathway selection appears to be controlled in part by phosphorylation of repair factors, but the underlying molecular mechanisms are unclear. Nbs1, which plays a key role in both DSB repair pathways, interacts with different response proteins in a phosphorylation-dependent manner.

Nbs1 is mutated in Nijmegen breakage syndrome (Carney et al., 1998; Varon et al., 1998), which is characterized by chromosomal instability, microcephaly, immunodeficiency, and a susceptibility to cancer. Nbs1 together with the endo/exonuclease Mre11 and the ATP binding protein Rad50 form